EXPERIMENTAL STUDIES ON THE PROLIFERATION OF THE YOSHIDA SARCOMA IN THE TISSUES UNDER ABNORMAL NERVOUS FUNCTIONS

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by

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I. INTRODUCTION

With regard to the tumor transplantation and tumor formation in denervated tissues, many experimental studies have been attempted, applying transplantable tumors or coal-tar to rabbits, mice, rats and fowls. But various writers reported divergent results[1, 2, 3, 4, 5] and there is no agreement with each other. KIMURA (1926)[6] pointed out that a cause of such a difference was the fact that different types of nerves or mixed nerves were cut and he clarified the acceleration of tumor growth in areas of sympathectomy. INOUE (1959)[7], of our school, transplanted the YOSHIDA sarcoma into the abdominal cavity of the rats after nerve block by the neurotoxic agent injection to the unilateral splanchnic nerves or to the renal plexus. He found that tumor formed in the kidney on the side the nerve was blocked. There is no other available reports about the relation between the visceral nerve blook and its influence upon the tumor growth ; therefore, the author reexamined INOUE's experiment and the results were the same as his. The author, furthermore, attempted a surgical section of the nerve to make nerve blook more decisive in the same type of experiment with the examination whether or not the surgical injuries had some influence on the tumor proliferation. Next, the author investigated tumor growth in denervated area by comparing the results of the section of intercostal nerve with that of the section of the autonomic nerves.

II. MATERIALS AND METHODS

Animals : Wistar-rats from the Inbred Animal Center of Kyoto University, 90g-150g in body weight, fed with mixed food and solid food for experimental animals produced by ORIENTAL Co.

Tumor : the YOSHIDA sarcoma from the laboratory of TAKEDA Pharmaceutical Co.

III. EXPERIMENT I

BLOCKING OF THE ABDOMINAL SYMPATHETIC TRUNK ON THE RIGHT SIDE WITH THE INJECTION OF 50% SOLUTION OF ALCOHOL

After anesthesia by intramuscular injection with 0.2-0.3 cc of 20 % solution of

urethane, 0.2 cc of mixed solution of pure alcohol and India ink in equal amounts was injected through the skin to the symathetic trunk in the site from 12th thoracic to 2nd lumbar vertebrae. Autopsy showed that the sympathetic trunk was stained with the India ink over 2-4 vertebrae in width. India ink sometimes stained peritoneum and subcutaneous tissue around it. From 5 to 17 days after the injection, about 0.1 cc of YOSHIDA sarcoma ascites was injected into the abdominal cavity with the glass capillary tube and autopsy was made after death from this tumor. To make complete degeneration[8], and to avoid an irritation reaction caused by the injection, transplantation was done 5-17 days after injection. after transplantation the length of survival days was from 5 to 13 days i. e. 8.3 days on average.

EXPERLMENTAL RESULTS : (Table 1)

Positive take occurred in 20 cases out of 23 (transplantation rate : 87%). Twenty-

1. Blocking of the Abdominal Sympathetic Trunk on the Right Side with the Injection of 50% of Alcohol

No.of rat	days between blocking and transplantation	survival days	ascites	omental tumor	take	renal tumor (r-side)
1	5	7	+	+	+	−
2	6	10	+	+	+	+
3	6	5	+	+	+	+
4	6	6	+	+	+	−
5	6	5	+	+	+	−
6	6	12	+	+	+	−
7	7	8	+	+	+	−
8	7	13	+	+	+	−
9	7	11	±	−	−	−
10	7	9	+	+	+	−
11	7	12	+	+	+	−
12	7	7	+	+	+	−
13	7	6	+	+	+	+
14	9	11	+	+	+	+
15	9	9	+	+	+	−
16	9	10	+	+	+	− block was incomplete
17	9	−			−	survived
18	10	8	+	+	+	−
19	10	9	±	−	−	−
20	10	7	+	+	+	− block was incomplete
21	17	8	+	+	+	+
22	17	6	+	+	+	−
23	17	7	+	+	+	−
		average: 8.3			20/23	5/18

the rate of lethal take : 20/23 (87%)
the rate of successful block : 21/23 (91%)
the rate of renal tumor formation : 5/18 (28%)

Fig. 1.

Renal tumor (right) and normal kidney (left) in the case whose right abdominal sympathetic trunk was blocked by alcohol injection. The black spot indicates the injected site.

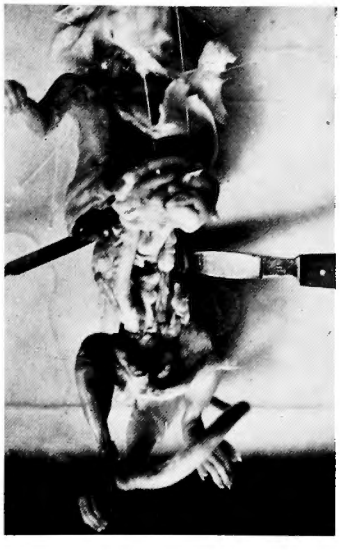


Fig. 2.

Vental aspect of Fig. 1.

one cases were injected accurately and the nerve stained well with India ink (successful block rate : 91%). Five cases out of 18 showed tumor only in the right kidney (Fig. 1, 2) and the rate of unilateral renal tumor formation was 5/18 (28%); this result was just the same as INOUE's. Other tumorous findings of the abdominal cavity, i. e. various degrees of tumor infiltration into omentum, pararenal fatty tissue, fatty tissue around the genital organs, were almost the same as in non-treated animals which died of the YOSHIDA sarcoma.

Histological Findings (Hematoxylin-Eosin Staining).

These tumors were composed of severely infiltrated pararenal fatty tissues, renal capsule and kidney and these three parts became a lump together. The renal capsule was partly strongly infiltrated and this infiltration spread itself to the kidney continuously (Fig. 3). And

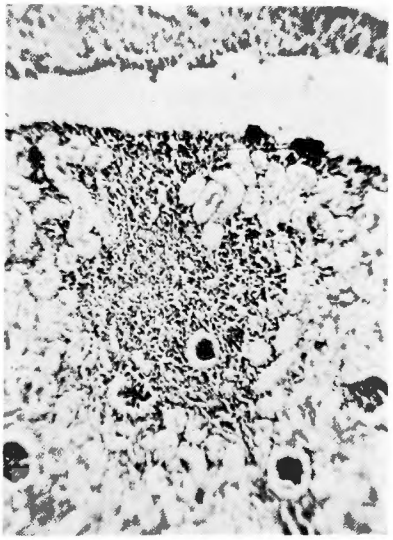


Fig. 3.

Microscopic view of renal tumor. Renal capsule has changed into a tumor mass (upper part) and tumor has infiltrated into the kidney continuously.

in the kidney, tumor cell infiltration spread from cortex to medulla continuously but sporadical infiltrative foci, suggesting hematogenic metastasis, was not found at all.

Degeneration of tubules and bleeding in the kidney were found at the strongly infiltrated parts but cell nuclei of the tubules still remained unchanged till late.

Kidney of the control side.

Macroscopically no renal tumor was found on the control side and even when infiltration was found in the pararenal fatty tissue, there were neither infiltration to the renal capsule nor clear metastasis in the kidney. Since autopsy was done after death from tumor, the hematogenic metastasis to both kidneys in a certain degree was expected, but metastasis to the control kidney was very slight or not found, though in a case with simple transplantation, hematogenic metastasis to heart, lung and kidney were found. The caudal lumbo-sacral sympathetic trunk was blocked also in the same way, but no tumor formation in the kidney was found at all.

From these results, block of the renal nerve seemed to play an important rôle in the tumor formation in the kidney in this experiment. It was necessary, however, to examine influences of injuries to the pararenal tissue caused by alcohol injection upon the tumor infiltration.

Ⅳ. EXPERIMENT Ⅱ

SECTION OF THE THORACIC SYMPATHETIC TRUNK

To exclude the influences of injuries to the pararenal tissue in consequence of injection, a section and blocking of the thoracic sympathetic trunk was tried. As the thoracic aorta runs down near the left thoracic sympathetic trunk, it is dangerous to attempt to cut it here, so section was done only on the right side. In this operation pleura, intercostal arteries, veins and nerves, back muscles and skin were injured but they had no close relation to the viscera. No signs of lung injuries followed this operation. Unilateral spontaneous pneumothorax occurred but none died of it and all recovered perfectly after 2-3 days, so the YOSHIDA sarcoma was transplanted intraabdominally in one group on the operation-day and, in another about 1 week thereafter.

METHOD

Hereafter NEMBUTAL intramuscular injection was used for anesthesia. In the right paravertebral part, fur was taken off and skin was disinfected with alcohol and mercurochrome. The sympathetic trunk which goes along the vertebral column was cut, stabbing with GRAEFE's knife through the skin to the vertebra and at autopsy success in section of the nerve was confirmed.

EXPERIMENTAL RESULTS : (Table 2)

Nine rats were operated on, 5-20 days after the operation tumor was transplanted. In one case out of nine nerve section was incomplete and the case was excluded. In 8 cases all died of tumor and their nerve section was sure but no renal tumor was found at all. In 12 cases which were trasplanted on the operation-day, 7 cases showed positive

2. Section of the Right Thoracic Sympathetic Trunk

No.of rat	days between section and transplantation	survival days	ascites	omental tumor	take	site of section	renal tumor (r-side)	
1	5	8	+	+	+	10 i.c.s.	−	
2	5	8	+	+	+	10	−	
3	7	10	+	+	+	10	−	
4	8	11	+	+	+	10	−	
5	8	14	+	+	+	8-10	−	
6	9	8	+	+	+	8	−	
7	9	10	+	+	+	8-10	−	※
8	20	7	+	+	+	10	−	
9	20	11	+	+	+	10	−	
10	the same day	8	+	+	+	7	−	
11		8	+	+	+	7	−	
12		3	−	−	−	6-7	−	**
13		11	+	−	−	obscure	−	※
14		3(killed)	±	−	−	8-9	−	**
15		24	+	+	+	9	−	
16		4	+	±	+	8	−	
17		4	+	−	−	7	−	
18		4	−	−	−	8	−	
19		7	+	+	+	10	−	
20		8	+	+	+	6	−	
21		8	+	+	+	7	−	

※ : section was incomplete
** : accumulation of pleural effusion
i. c. s. : intercostal space

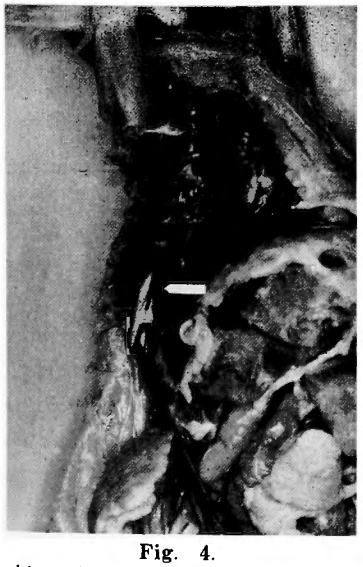


Fig. 4.
Blocking of the right thoracic sympathetic trunk. The black spot indicates the injected site.

Fig. 5.
Splanchnic nerves and nerve branches around the pararenal body.

take but no renal tumor was found. In 13 cases whose thoracic sympathetic trunk was blocked with alcohol injection and then transplanted, no renal tumor was found.

It was concluded that the YOSHIDA sarcoma transplantation after the section or blocking of thoracic sympathetic trunk was not followed by the tumor formation in the kidney, and hematogenic metastasis was not found at the injured site. The reason seems to be as follows. Bilateral sympathetic trunk are connected with many nervous branches and greater and lesser splanchnic nerves, composed of the sympathetic nerve of the same side, run into one celiac ganglion from which branches enter the kidney; it is supposed naturally that, even with this injury, the renal plexus must have kept its function as usual.

V. EXPERIMENT Ⅲ

SECTION OF GREATER AND LESSER SPLANCHNIC NERVES

In rats it is difficult surgically to destroy celiac or renal plexus solely. The author tried to make degeneration of them by use of silver nitrate solution but its corrosive action extended itself too widely to destroy the nerve selectively. In Experiment Ⅱ, to avoid the injury of the diaphragm, section was done at so high a level that the lesser splanchnic nerve fibers might be left intact. Material fixed with formalin shows clearly that the splanchnic nerve goes through the capsular tissue of the suprarenal body (Fig. 5), as the section was tried here. Rats have accessory suprarenal body and, by its compensation, signs of insufficiency of the suprarenal gland hardly appears even if unilateral suprarenal body was extirpated[9].

METHOD

In the back at the level of the kidney an incision was made, the suprarenal body with its capsule was extirpated from surrounding tissue and replaced where it was. On another side, an incision was made and the suprarenal body was observed directly but left untouched, as control. The splanchnic nerve was severed by extirpation of the suprarenal body, then the abdomen was closed. From 14 to 24 days after this operation the YOSHIDA sarcoma was transplanted.

EXPERIMENTAL RESULTS: (Table 3, 4)

Various degrees of tumor infiltration were observed in the bilateral pararenal fatty tissues. In some cases renal capsule and kidney were also infiltrated with tumor and these three parts became a lump together but in others only pararenal fatty tissues were infiltrated and kidney was easily freed from the renal capsule and no infiltration was found in the kidney. In the present experiment, the object of the search was the kidney tumor strictly; that is to say, infiltration of pararenal fatty tissues was distinguished from renal tumors, since they represented a finding common to the intraabdominal tumor transplantation.

In this experiment renal tumor formation was observed in ratio of 5/15 (33%) on the left side (operated-side), and 4/14 (29%) on the right side (operated-side) after the

3. Section of the Left Splanchnic Nerve

No.of rat	days between section and transplantation	survival days	ascites	omental tumor	ranal tumor (l-side)	renal tumor (r-side)
1	7	5	+	+	−	−
2	7	6	+	+	−	−
3	7	7	+	+	+	−
4	8	10	+	+	−	−
5	13	9	+	+	−	−
6	14	8	+	+	−	−
7	14	8	+	+	+	−
8	17	9	+	+	−	−
9	17	9	+	+	+	−
10	17	14	+	+	−	−
11	19	9	+	+	−	−
12	19	11	+	+	−	−
13	24	11	+	+	+	−
14	24	12	+	+	−	−
15	24	17	+	+	+	−
		average : 9.7			5/15	

the rate of the renal tumor formation on the left left side : 5/15 (33.3%)

4. Section of the Right Splanchnic Nerve

No.of rat	days between section and transplantation	survival days	ascites	omental tumor	renal tumor (r-side)	renal tumor (l-side)
1	6	15	+	+	−	−
2	6	7	+	+	−	−
3	6	13	+	+	−	−
4	7	8	+	+	−	−
5	8	8	+	+	+	−
6	12	9	+	+	−	−
7	12	9	+	+	+	−
8	22	8	+	+	−	−
9	24	7	+	+	−	−
10	24	7 (killed)	+	+	−	−
11	24	8	+	+	−	−
12	24	8	+	+	+	−
13	24	9	+	+	+	−
14	24	13	+	+	−	−
		average : 9.4			4/14	

the rate of the renal tumor formation on the right side : 4/14 (28.5%)

unilateral splanchnectomy. Compared with INOUE's result caused by splanchnic nerve block with Irgapyrin injection, i. e., 5 %, this result indicates a rather higher rate of tumor formation and the difference seems to be due to a more direct method of nerve section employed here.

Ⅵ. EXPERIMENT Ⅳ

METASTASIS TO THE OPERATIVE WOUND IN THE ABDOMINAL WALL

To examine the possible influence of operative wounds near the kidney upon the metastasis to the kidney in the experiment V, this experiment was undertaken.

METHOD

In the abdominal wall of 9 rats in experiment Ⅲ, an incision of ca. 3 cm in length to the peritoneum was added medially and metastasis to the wound was examined after transplantation.

EXPERIMENTAL RESULTS

In 6 cases out of 9, metastasis to the wound was found and in 5 cases out of these 6, metastasis was produced through the omentum which adhered to the wound and, in the remaining one, metastasis extended continuously from a tumor of the abdominal wall where tumorous ascites were injected. On the other hand, in 3 cases without metastasis no adhesion of omental tumor but only suture string and scar tissue in the wound were clearly observed (Fig. 6). In a case with omental adhesion, metastasis grew crossing the operative wound, so the adhesion seemed a more predominant factor for metastasis to peritoneum rather than the operative wound itself. Even in the case transplanted 1 month after the operation, when wound already changed into scar tissues, this tendency was also noted and the operative wound itself did not form the site of predilection to metastasis.

In the histological picture of the wound, the peritoneum remained almost normal and no disseminated foci in and around the blood vessels were found, but there was infiltrative expansion into subperitoneal tissue from the wound of the peritoneum (Fig. 7, 8). So metastasis seemed to be continuous in nature rather than via blood vessels.

Very few leukocytes were found in the site of the tumor infiltration. Around the suture string leukocytes and fibroblasts gathered together (Fig. 9, 10), and almost no tumor cells were found. That is to say, in the site of the leukocytic infiltration caused by inflammatory reactions, invasion of tumor cells seemed to be hindered. Sometimes subcutaneous tumor was found at the inoculated site

Fig. 6.

Peritoneal wound without omental adhesion. Only suture strings are seen.

Fig. 7.

Microscopic view of peritoneal wound. Subperitoneal tumor infiltration is seen.

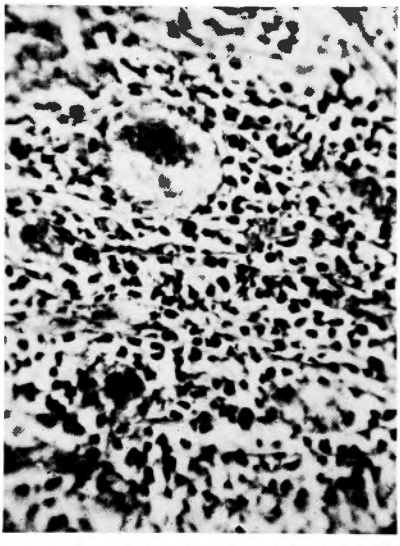


Fig. 8.

Magnified picture of Fig. 7. In the blood vessel only blood cells are seen.

in the abdominal wall but it did not become so large as when transplanted subcutaneously.

In the primary healed wound in this experiment, tumor metastasis was hindered evidently. That is, the wound in its healing process will hardly form a site of predilection to metastasis. Though we often found metastatic tumor at the postoperative wound of tumor-patients clinically, the wound itself did not form a site of predilection to metastasis when tumor was transplanted after primary healing of laparotomy in rats experimentally.

From these findings, it is thought that a wound around the kidney does not exert

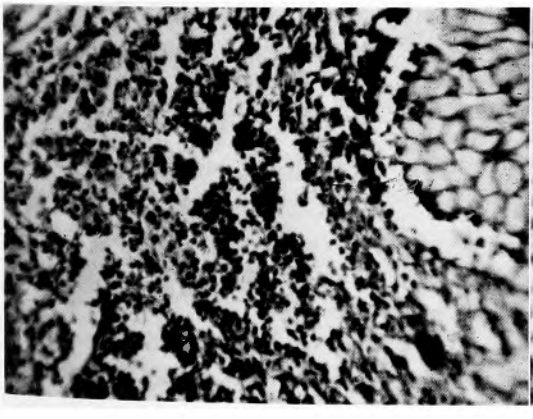


Fig. 9.

Leukocytic infiltration around the suture string.

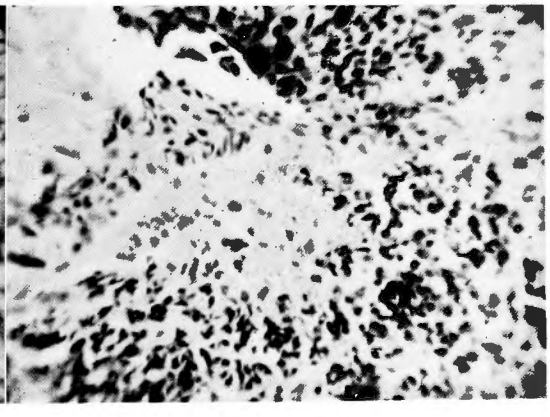


Fig. 10.

A section of the suture string (in left upper part) is surrounded by fibroblasts and bleeding zone. Outside of them tumor cells are seen.

an accelerating action on the formation of metastatic renal tumor in the above experiment. The next problem is whether the possible change in the peritoneal permeability caused by the operation is influential on the tumor proliferation or not.

Ⅶ. EXPERIMENT Ⅴ

ABSORPTION OF INDIA INK INJECTED INTO THE ABDOMINAL CAVITY AFTER THE LAPAROTOMY OR THE OPERATION FOR SPLANCHNIC NERVE SECTION

To know the degree and site of predilection for absorption of particulate matters, intrabdominal India ink injection method was adopted.

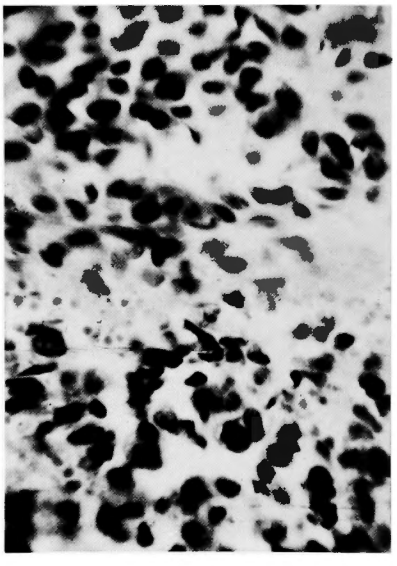


Fig. 11.

Magnified picture of Fig. 10. From top downwards, zone of fibroblasts, that of bleading and that of tumor cells.

METHOD AND RESULT

India ink, 1 cc per 100g body weight of rat, was injected into the abdominal cavity, then the rat was killed by hemorrhage and autopsied. As a control, untreated rats were injected with India ink and the site of its absorption was investigated.

India ink spot was most predominant in the omentum and it was noticed in paragenital fatty tissues, mesenterial attachment of bowels and pararenal fatty tissues, too. Compared with other authors' findings concerning the diaphragm of rabbits and mice[10, 11, 12], India ink absorption was rather poor in rats. These sites quite coincided with areas of predilection to metastasis of the YOSHIDA sarcoma in rats, so the tumor cells seem to be passively absorbed to these sites rather than to invade the tissue actively.

India ink injection was done in the same cases as in experiment Ⅲ, whose left splanchnic nerve was cut at the site of the suprarenal body with a control incision on the right side. They were killed and autopsied 1.5, 18, 48 hours after injection. In these cases ink spot was rather slighter at the operated sites, too, and there was no difference between these 3 cases. That is, the degree of absorption was not related to the time intervening and the incised peritoneal wound did not show predominant absorption. In all cases no evident absorption was observed at the surface of the kidney. With these findings, the renal tumor such as observed in the experiment above was not caused by the possible hyperabsorbability at the operated wound. In the rat with primarily closed wound after laparotomy such as in experiment Ⅳ, a little ink spot was observed at the peritoneal wound.

From the experiment above mentioned, the formation of metastasized renal tumor due to the YOSHIDA sarcoma, transplanted into the abdominal cavity after the section of

renal nerves, does not seem to depend on the conditions brought about by non-specific surgical injuries.

Ⅷ. EXPERIMENT Ⅵ

SUBCUTANEOUS TRANSPLANTATION AT THE ABDOMINAL WALL AFTER THE SECTION OF INTERCOSTAL NERVES

It is well known that there occurs atrophy of epithelium and muscles after the denervation of supplying spinal nerves, and there are many experimental reports about the relationship between tumor growth and the section of sciatic nerve. In the present paper the author examined the manner of the tumor growth in areas of intercostal nerve section and compared the results with that of the autonomic nerve section.

METHOD

Over the disinfected back of anesthetized rats, the 11th-13th intercostal nerves were resected and only a control skin incision was made on the other side. After the operation the wound was primarily closed. The rats with this treatment were divided into 3 groups. Group I was transplanted on the operation-day and group Ⅱ and group Ⅲ were transplanted about a week and about a month after the operation, respectively.

From 0.05 to 0.1 cc of YOSHIDA sarcoma ascites was injected in equal dose subcutaneously at the region of the denervated abdominal wall and at the opposite site as control. Hard indurations at those sites were observed 3-4 days later and they developed to the subcutaneous tumors about a week later; these tumors were measured in length and width. The results were as shown in Table 5,6. These tumors, in cases of positive take, became larger gradually and sometimes both were connected into a dumb-bell shape. An ulcer formed over the surface of the tumor.

In the peritoneal cavity, omental tumor and ascites were also observed and at last the rats died of the YOSHIDA sarcoma. The length of survival period were far longer in these cases than that of the control with simple intraabdominal transplantation without any other previous treatment.

EXPERIMENTAL RESULTS : (Table 5, 6)

In groups Ⅱ and Ⅲ, tumor growth was less evident in the denervated area than in the control side regardless of the side of denervation. In group I, no influence of denervation upon the tumor growth was observed in general. In other words, when the subcutaneous transplantation was made after the completion of degeneration of the intercostal nerves, tumor growth seemed to be retarded on the operated side as compared with the control side.

Ⅸ. EXPERIMENT Ⅶ

METASTASIS TO THE PERITONEUM SUTURED WITH OMENTUM

In Experiment Ⅵ, peritoneal metastasis which was continuous from the adhered

5. Unilateral Section of the 11th-13th Intercostal Nerves with Simultaneous Subcutaneous Transplantation in the Abdominal Wall
The size of the tumor was represented by length and width.

Section in the right side		Section in the left side	
right-tumor	left-tumor (control)	right-tumor (control)	left-tumor
30×30 >	13×13	10×10 >	5× 4
8× 8 <	12× 8	12×10 <	17×12
8× 8 >	0	10×10 <	18×10
10×10 <	10×12	10× 8 <	12× 8
15×13 >	12×10	9× 9 >	9× 8
0 <	5× 5	10×10 >	9× 7
5× 5 <	10× 5		
15× 7 >	10× 8		
the rate of retarded growth on the severed side	4/8 (50%)	3/6 (50%)	
Transplantation was done a week after section of the nerves.			
6× 4 <	12× 7	0	0
0 <	10× 8	0 <	12× 8
12× 8 <	13×13	15× 9 >	10× 7
		9× 9 >	9× 6
the rate of retarded growth on the severed side	5/6 (83%)		
Transplantation was done about a month after section of the nerves.			
15×10 =	15×10	8× 7 <	13×10
17×12 <	15×15	15× 9 >	12×10
10×10 <	15×10	11× 7 >	9× 5
12×10 <	13×10	15× 9 >	12×10
12×10 <	15×13	5× 4 =	5× 4
the rate of retarded growth on the severed side	7/10 (70%)		

omental tumor was observed. The author hoped to make a peritoneal metastasis at any site and at his will by allowing tumor cells to pass through the sutured portion of omentum to the abdominal peritoneum artificially. Omentum is most absorbable of India ink and, when YOSHIDA sarcoma ascites are used, this tissue is most likely to form tumors. With this fact in mind the author aimed at the examination of the relationship between the intercostal nerve supply and peritoneal metastasis produdced in this way.

METHOD

Laparotomy was made medially and the margins of omentum were sutured with the

6. Suturing of the Omentum to the Abdominal Peritoneum

A. Unilateral Section of the Intercostal Nerves

No.of rat	severed intercostal nerve	days between section and transplantation	survival days	ascites	omental tumor	metastasis to the sutured site	
						right	left
1	r. 12th, 13th	7	8	+	+	—	—
2	r. 12th, 13th	7	11	+	+	size of a rice grain	—
3	r 12th, 13th	8	11	+	+	—	—
4	r. 11th-13th	7	10	+	—	—	—
5	l. 12th, 13th	7	12	+	+	—	*
6	l. 11th-13th	8	11	+	+	—	—
7	l. 11th-13th	7	10	+	+	—	—
8	l. 11th-13th	7	10	+	+	—	—

* : Continuous metastasis from the site which the ascites were injected.

B. CORTISONE injection at the sutured site

No.of rat	injected side	days between operation and transplantation	survival days	ascites	omental tumor	metastasis to the sutured site	
						right	left
1	right	7	6 (killed)	+	+	—	—
2	right	7	7	+	+	—	—
3	right	7	8	+	+	—	—
4	right	7	11	+	+	—	—
5	left	7	5	+	+	—	**
6	left	7	8 (killed)	+	+	**	**
7	left	7	12	+	+	—	—
8	left	7	10	+	+	—	—
9	both	7	8	+	+	—	—
10	both	7	8	+	+	—	—

* * : Induration due to tumor infiltration

peritoneum of the left and right side of abdmen and the abdominal wall was primarily closed. 11th to 13th intercostal nerves on one side were resected at the back and a control skin incision was made on the other side. Intraabdominal transplantation of tumor was made after about a week.

EXPERIMENTAL RESULTS

Findings in the peritoneal cavity caused by the YOSHIDA sarcoma were almost the same as in the case without treatment and omental tumor also formed but, contrary to expectation, continuous metastasis from omental tumor to the peritoneum through the suture-site was not observed at all (Fig. 13). It was suspected that inflammatory reactions at the suture-site hindered the metastasis; so, 0.04 cc of cortisone acetate was injected at the suture-site for 10 cases to prevent the possible inflammatory reaction and then transplantation was done in the same way. In 2 cases out of 10, only small induration was observed at the suture-site but no evident metastatic tumor was found at all. Histologically,

Fig. 12.

Section of the right splanchnic nerve was performed around the right pararenal body. A week later India ink was injected intraabdominally and 1.1/2 hours later the rat was killed and autopsied. Atrophied pararenal body is seen near the kidney but in the operative wound no evident absorption of India ink is noted.

Fig. 13.

Control side of Fig. 12.
Pararenal body of normal size is seen on the kidney and in the operative wound near the kidney faint ink spots are seen. Omentum is tinted evidently.

omental tumor was located apart from the peritoneum and no tumor cell infiltration was noticed into the subperitoneal tissue (Fig. 14). In the border of the peritoneum and the omental tumor, leukocytic infiltration was poor and fibroblasts were observed. That is, no evident tissue reaction was observed in the region of tumor cell infiltration but fibroblasts were found where there was healing process in the suture-wound.

Thus, relationship between tumor growth and intercostal nerve supply could not be examined. But in this experiment the author confirmed that postoperative inflammatory tissues would not form *locus minoris resistentiae* to the infiltration of the YOSHIDA sarcoma. In the former experiment, a small quantity of India ink was absorbed through the peritoneal wound but in the case of tumor transplantation no metastasis was found at the site. It seemed, therefore, that an unknown force, hindering metastasis, occurred in tissue reaction and this protected the operative wound from tumor infiltration in spite of an increased permeability of the peritoneum. If we assume that some tumor cells had grown in the abdominal cavity by the time of the laparotomy, tumor cells would infiltrate into the fresh wound tissue and that tumor cells injected into the abdominal cavity after wound healing of the laparotomy could hardly infiltrate the wound tissue, the phenomenon of tumor metastasis to the postoperative scar in tumor patients is likely to be due to the scattering of tumor cells during the surgical operation.

X. DISCUSSION

INOUE, of our school, found the unilateral renal tumor formation in rats transplanted

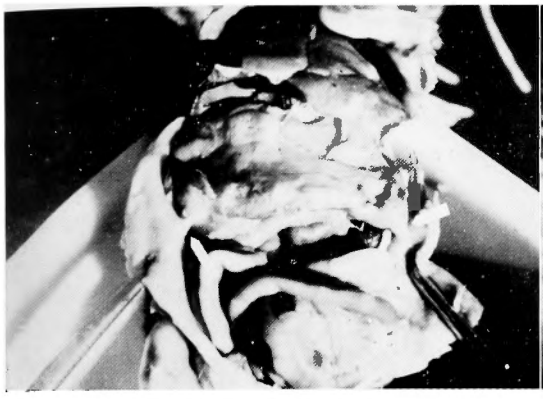


Fig. 14.

Omentum sutured with the peritoneum and omental tumor. This tumorous change stopped at the sutured site.

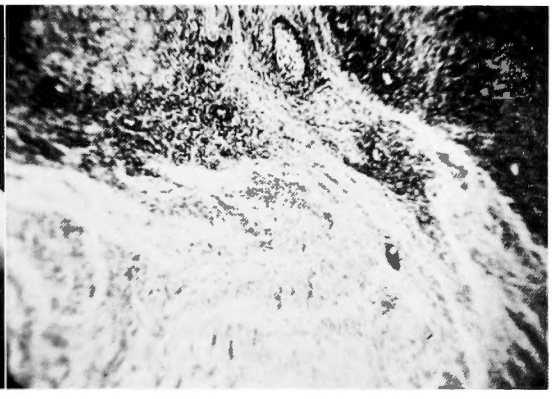


Fig. 15.

Microscopic view of the sutured site shown in Fig.14. The uppor half, omental tumor; lower half, cicatricial connective tissue.

with the YOSHIDA sarcoma after blocking of unilateral renal nerve plexus or sympathetic nerve trunk and he concluded that renal nerve degeneration would make the kidney a favorite site of tumor growth. The present author cut these nerves before transplantation and got the same result. Moreover, he examined influences of the injuries upon the tumor formation and reached the conclusion that renal sympathetic nerve section would aid the renal tumor formation. The fact of an increased tumor growth in areas removed from the sympathetic nerve supply agrees with that of KIMURA (1926) who tried similar experiments, using the ears of rabbits and rabbit tumor.

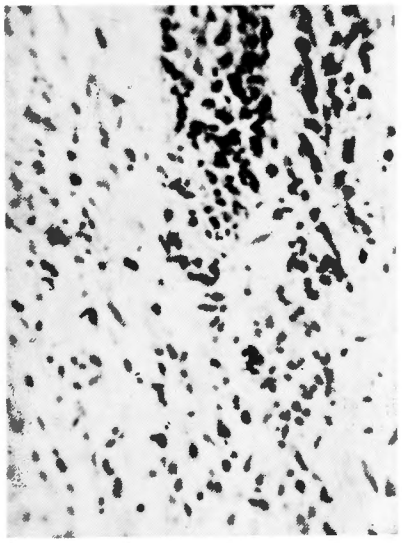


Fig. 16.

Magnified picture of Fig. 15. The focus of tumor infiltration is capped by fibroblasts chiefly.

About the physiology of renal nerve much is yet unknown and obscure except the fact of the sympathetic, vasoconstrictor action. In the present paper, however, histological findings of increased hematogenic metastasis was not observed after this operation; only resistance against contact metastasis seemed to be decreased.

JUNGMANN and BERNHARDT reported on the decreased resistance of the denervated kidney against toxin and pathogenic bacteria[14]. The author also should like to think of special actions of sympathetic nerve against tumor.

It is an interesting problem clinically whether operative wound forms the site of predilection to metastasis or not. To examine this problem, omentum was sutured to the

peritoneum and then the YOSHIDA sarcoma was transplanted. Omentum carried almost always evident tumors but metastasis to the peritoneum was stopped or hindered at the suture-site, while metastasis to peritoneum was evident when spontaneous omental tumor adhered to peritoneum in control rats. Rats with primarily sutured peritoneal wound was transplanted with the YOSHIDA sarcoma and operative wound was examined microscopically. Around the suture string fibroblasts and leukocytes were found and tumor cell infiltration was hindered. From these facts, it was concluded that inflammatory tissue reaction hindered the tumor infiltration in normal rats.

Clinically, metastasis to an operative wound was often noticed. From the author's point of view, this seemed to be due, on the one hand, to abnomal internal circumstances of tumor patients, and, on the other, to scattering of already existing tumor cells during operation.

ASCHNER, FUJINAWA[15], KATO, SUEYASU, FUJINAMI and KIMURA reported on an increased tumor growth in the area where the motor and sensory nerve supply was severed; especially in KIMURA's experiment, no influences were observed when rabbit sarcoma was transplanted to rabbit's ear on the same day of the section of spinal nerve, while growth was hindered when tumor transplantation was done about a week after the nerve section. The experiment of intercostal nerve section in this paper agrees with KIMURA's result.

XI. CONCLUSION

Proliferation of the YOSHIDA sarcoma at tissues of abnormal nervous function was studied and the following results were obtained.

1) Before the intraabdominal transplantation of the YOSHIDA sarcoma to Wistar-rats, unilateral splanchnic nerve section or abdominal sympathetic trunk block was made and renal tumor formation was observed on the denervated side, while no renal tumor formation was found when thoracic sympathetic trunk was cut or blocked.

2) Influences of possible accessory injuries following the operation of nerve section on the tumor growth was examind and no accelerating action on the tumor growth was observed; therefore, the section of these nerves seems to be most responsible for the renal tumor formation. Histologically, findings of increased hematogenic metastasis was absent and special action of sympathetie nerve to tumor was suggested.

3) Postoperative tissue reaction in normal rat hinders metastasis of the YOSHIDA sarcoma transplanted after the operation.

4) When the YOSHIDA sarcoma was transplanted subcutaneously in the abdominal wall after unilateral intercostal nerve resection, tumor growth was retarded on the denervated side, while, when the tumor was transplanted on the day of the nerve resection, no influences of denervation on the tumor growth was observed.

5) From these results it was concluded that the operative wound itself did not form the site of predilection to metastasis when tumor was transplanted postoperatively; scattering of the tumor cells during operation seems to be necessary for the development of a wound metastasis.

EXPERIMENTAL STUDIES ON THE EFFECT OF "DFP" UPON THE PROLIFERATION OF THE YOSHIDA SARCOMA

Ⅱ. INTRODUCTION

In this paper the degree of the proliferation of the YOSHIDA sarcoma was studied when the cholinergic nerve tone was made dominant by injection of a large dose of DFP (Diisopropyl fluorophosphate), an anticholinesterase drug.

Ⅱ. PRELIMINARY EXPERIMENT Ⅰ (Table 1)

MEASUREMENT OF CHOLINESTERASE VALUE IN NORMAL RATS

To 21 rats weighing 60-220 g, LD_{50} of DFP (3 mg/kg for subcutaneous injection, 1 82 mg/kg for intramuscular injection)[16] was injected and the relation between the body weight and the occurrence of death by the poisoning was examined. The serious degree of poisoning caused death of the animal squatting down with muscular twitching, epiphora and exophthalmia, while slight one permitted survival and recovery though the rats showed similar symptoms and they were dirty and dystrophic on account of diarrhea.

Since many died in a group and many were alive in another after the injection, influences of the body weight, method of the injection and the effect of the season in which the experiment was carried out were examined; however, no constant correlation was found among them. This seems to be due to the fact that members of a group were composed of brothers and sisters mostly, hence variable hereditary characteristics with group. To clarify such characteristics, the cholinesterase (ChE) level of the serum of some untreated rats was measured with WARBURG's manometer after AMMON's method[17] and evident differences were found among animals. For the sake of convenience, they were divided into two groups, i. e., high or low in terms of the ChE level.

While the range of ChE in normal human serum is from 50 to 90 CO_2 cmm/ml min[18]., that of normal rats is from 24.3 to 43.7 CO_2 cmm/ml min. and the ratio of

1. Injection of DFP in Dose of LD_{50} (Subcutaneous Injection of 3mg/kg and Intramuscular Injection of 1.82mg/kg)

route of injection	cases	body weight	cases died of poisoning	survivals
subcutaneous	6	about 90g	6	0 *
subcutaneous	3	185g-220g	3	0 **
subcutaneous	5	100g-220g	1	4 ***
subcutaneous	4	150g-170g	0	4
intramuscular	3	60g- 80g	0	3
		total	10/21	11/21

* : Three cases died within one hour, other 3 died by the next day.
** : Two cases died in about one hour, other one died by the next day.
*** : One case died on the 2nd day.

the maximum to minimum level is almost the same as the human's. The fact that ChE value of rats is lower than that of a human is thought to be due to the reason that ChE contents of serum is parallel with Albumin contents of serum and that A/G in rats is 1.4,[19] while it is 1.5-2.5 in human.[20]

Ⅲ. PRELIMINARY EXPERIMENT Ⅱ (Table. 2).

MEASUREMENT OF CHOLINESTERASE VALUE AFTER "DFP" INJECTIONS

As some rats died of acute poisoning with a single subcutaneous injection of 2mg/kg of DFP and some survived even after 4 injections given in the same dose every other day, serum ChE was measured in cases to which DFP was injected from 1 to 4 times; drop in the ChE value was examined.

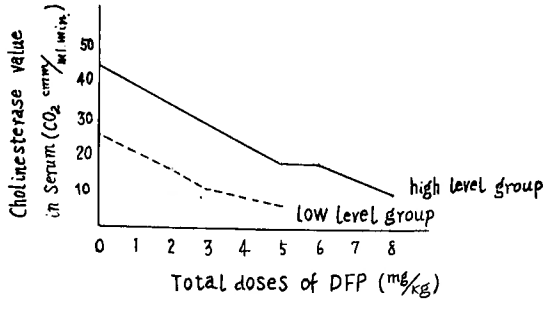
MATERIALS AND METHOD

Serum was separated from blood collected by heart punction and diluted with RINGER's solution in 50 folds and the ChE was measured with WARBURG's manometer

2. Cholinesterase Value in Serum (CO_2 cmm/ml.min.)

	control	DFP 2mg/kg		DFP 2mg/kg and 1mg/kg	DFP 2mg/kg and 3 times of 1mg/kg	DFP 3 times of 2mg/kg	DFP 4 times of 2mg/kg
high level group	43.7				18.0	20.3	14.0
	43.68					20.0	10.8
						18.2	7.3
						12.9	7.25
average	43.7				18.0	17.9	9.84
		s.i.	i.i.				
low level group	25.5	22.0	14.6	14.6	10.75		
	24.3	13.1	13.0	10.56	2.76		
		11.7	8.7	7.31			
average	24.9	15.6	12.1	10.82	6.76		

s.i. : subcutaneous injection
i.i. : intraabdominal injection



after AMMON's method. ChE in serum was represented by CO_2 gas volume in cmm produced per ml of serum per minute. As Acetylcholine preparation, OVISOT (DAIICHI Pharmaceutical Co) was used.

EXPERIMENTAL RESULTS

1) Control group

Out of 4 normal rats 2 gave high and the other 2 low level; in other words, a half belonged to the group of high level and the other half, to the group of low level.

2) A single subcutaneous injection of 2mg/kg of DFP

Six cases were injected 2 days previously and the serum was obtained from 3 cases. The other 3 cases were injected with 2mg/kg more and they died of poisoning. All the members of this group seemed to die after being injected twice with 2mg/kg of DFP each time,; i. e., they belonged to the low level group.

Three cases were injected with 2mg/kg of DFP intraabdominally and their ChE value was found to be lower than in the cases of subcutaneous injection given in the same dose of DFP.

3) Subcutaneous injections of 2mg/kg and 1mg/kg of DFP every other day.

One case out of 7 died of poisoning only with 2mg/kg of DFP. On the 2nd day after this injection, cases of the survival were injected further as follows: 4 cases with 1mg/kg, and 2 cases with 2mg/kg were injected; the latter animals died of poisoning. Members of this group could endure 2mg/kg plus 1mg/kg of DFP injections but could not endure 2 injections of 2mg/kg; therefore, they belonged to the low level group. (Owing to hemolysis, data of a case were discarded.)

4) Subcutaneous injections of DFP in dose of 2mg/kg plus 3 subcutaneous injections of it in dose of 1mg/kg.

Injections were done 4 times in total every other day. ChE value of a case was higher than in 3), so it belonged to the high level group; the other 2 belonged to the low level group.

5) Three subcutaneous injections of DFP in dose of 2mg/kg every other day.

Six cases died all after 3 injections; another 5 cases survived all and 4 out of them were used for measurement. These 5 cases were thought to belong to the high level group, because the total does of DFP was 6mg/kg and this group not only could endure 3 injections but also showed higher value than did 3) or 4).

6) Four subcutaneous injections of DFP in dose of 2mg/kg every others day.

To the surviving cases which stood the procedure of 5), an additional 2mg/kg was injected; 2 days later ChE level was measured.

With this classification in mind, the relation between the drop of serum level of ChE and the total dose of DFP was examined graphically. The slopes of the drop in two graphs were about the same; that is, the rate of the decrease of serum ChE level by the DFP injection was almost the same irrespective of whether rats could endure repeated injections of DFP or not; the rate of the decrease depend not on the sensibility to DFP but on the ChE level before treatment. (Of course there must be middle level group, because some rats died of poisoning wiith various dose of DFP between 2mg/kg

and 8mg/kg in total.)

From these results the author reasons as follows: since there is a considerable individual variation with regard to ChE contents in the serum of rats independent of body weight and season, conclusion must be drawn from observations on a group consisting of animals with the same level of ChE value or on large group, i. e., containing as many cases as possible.

Ⅳ. EXPERIMENT I

INFLUENCES OF "DFP" INJECTION ON PROLIFERATION OF THE YOSHIDA SARCOMA

DFP in dose of 2mg/kg was injected subcutaneously or intraabdominally every other day from 1 to 4 times in a group consisting of about 5 rats and, 2 days after the injection, the YOSHIDA sarcoma was transplanted into the abdominal cavity. These experiments were repeated twice or thrice to confirm the observation.

EXPERIMENTAL RESULTS (Table. 3).

That a death was due to the YOSHIDA sarcoma was determined when there was found the formation of omental tumor and accumulation of tumorous ascites; all cases which died within 4 days after transplantation were excluded, because in these cases intraabdominal findings were too poor to ascribe the death to the tumor, and because death might have been caused by DFP poisoning or some accident due to unfavorable circumstances to life. Many died when DFP was injected 3 or 4 times in does of 2mg/kg, so additional injections were made only with 1mg/kg of DFP in some groups.

1) A single subcutaneous injection in dose of 2mg/kg of DFP.

The rate of lethal take of the tumor was 9/12 (75%), i. e., the same as the control

3. Subcutaneous injection of 2mg/kg of DFP

No.of rat	survival days	ascites	omental tumor
1	10	+	+
2	11	±	+
3	11	+	+
4	12	+	+
5	12	−	−
6	13	+	+
7	14	−	−
8	8	+	+
9	9	+	+
10	9	+	+
11	10	+	−
12	alive		

average survival days:10.3days
rate of lethal take:9/12 (75%)

Intraabdominal injection of 2mg/kg of DFP

No.of rat	survival days	ascites	omental tumor
1	4	−	−
2	6	+	+
3	6	+	+
4	7	+	+
5	7	+	+
6	10	+	+
7	7	+	+
8	8	+	+
9	8	+	+
10	9	+	+
11	10	+	+

average survival days:7.8days
rate of lethal take:10/11 (91%)

Two subcutaneous injections of 2mg/kg of DFP

No.of rat	survival days	ascites	omental tumor
1	9	+	+
2	9	±	+
3	10	+	+
4	12	−	−
5	16	+	+
6	10	+	+
7	10	+	+
8	9	+	+
9	alive		

control (without tumor transplantation)

10 alive

11 alive

average survival days:10.4days

rate of lethal take:7/9 (78%)

group. The possible ChE value in the high level group after injection of DFP in dose of 2mg/kg was deduced to be about 30 CO_2 cmm/ml min. from table 2, and it remined still higher than that of the untreated low level group. For these reasons, effect of DFP in this dose level seems to be negligible for the tumor transplantation.

2) The rate of lethal take was 10/11 (91%) and average survival days were 7,8 days when 2mg/kg of DFP was injected into the abdominal cavity. ChE value became lower than that of the group of subcutaneous injection given in the same dose.

3) Two subcutaneous injections of DFP in dose of 2mg/kg every other day.

Five cases out of 6 died of poisoning in a group. In another one, 9 cases out of 11 which survived these injections were transplanted with the tumor and 7 cases out of these 9 died of tumor. As control, 2 cases were not transplanted and they survived the DFP treatment. This group belonged to the high level group.

One subcutaneous injection of 2mg/kg and two subcutaneous injections of 1mg/kg of DFP

No.of rat	survival days	ascites	oment l tumor
1	died of poisoning		
2	died of poisoning		
3	7	+	+
4	16	+	+
5	16	+	+
6	alive		
7	died of poisoning		
8	died of poisoning		
9	died of poisoning		
10	10	+	+
11	10	+	+
12	10	+	+
13	10	+	+
14	13	+	+

average survival days:11.5days

rate of lethal take:8/9 (89%)

Four subcutaneous injections of 2mg/kg of DFP

No.of rat	survival days	ascites	omental tumor
1	4	+	+
2	5	+	+
3	6	+	+
4	6	+	+
5	7	+	+
6	6	+	+
7	6	+	+
8	6	+	+
9	8	+	+
10	8	+	+
11	8	+	+
12	8	+	+

control (without tumor transplantation)

13 died of poisoning

14 alive

15 alive

16 alive

17 alive

average survival days:6.5days

rate of lethal take:12/12 (100%)

4) One subcutaneous injection of DFP in dose of 2mg/kg plus 2 subcutaneous

injections in dose of 1mg/kg every other day.

Five cases out of 14 died of poisoning and the rest were transplanted and they died of tumor with a high death rate.

In these groups which were injected with a total of 4mg/kg such as shown in 3) and in 4), it seems that members of the low level group died of poisoning and these of the high level group died of tumor.

In spite of the reduction in the ChE level in the high level group, as the graph shows, no shortening of the survival days was noted in this group; it appears that the group of the high level relatively resists the death from tumor.

5) Four subcutaneous injections of DFP in dose of 2mg/kg every other day.

In a group, all of 6 cases died of poisoning after 3 injections, and, in the other 2 groups, one case out of 17 died of poisoniog, and, 12 cases out of the remaining 16 were transplanted after 4 injections and all died of tumor with a shortened survival period. In this group typical intraabdominal findings were observed even in a case which died on the 4th day after transplantation, and evident omental tumor with accumulated tumorous ascites were seen also in the rest. Four cases which survived after the injections and were not transplanted were observed the rest of their lives as control and they did not die of poisoning since.

The cases injected with 2mg/kg of DFP intraabdominally and the cases treated with more than 2 injections of 2mg/kg of DFP subcutaneously died with a high mortality in general, and findings in the abdominal cavity were also evident. When this mortality, 37/41 (90%), was compared with that of the untreated, 177/237 (75%), difference was significant, i. e., $\frac{d}{\sigma d}=2.74^{\cdot\cdot}>2$ $x^2=4.77^{\cdot\cdot}>4$ $p<0.039$. It was concluded, from table 2, that the rate of lethal take increased when ChE value was lower than about 14.6 CO_2 cmm/ml min.

V. EXPERIMENT II

EFFECT OF "DFP" UPON THE NEGATIVE-TAKE-GROUP

In a negative-take-group which survived more than 4 weeks after transplantation and where no accumulated ascites were found by puncture, some were injected with 2mg/kg of DFP intraabdominally and the others were treated with 4 subcutaneous injections of 2mg/kg of DFP every other day. The YOSHIDA sarcoma was transplanted 2 days after these injections. These doses were sufficient to depress the ChE value in serum surely.

EXPERIMENTAL RESULTS

4. Rate of lethal take of the YOSHIDA sarcoma in each month.

month	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Total
rate	10/13	12/16	26/34	25/34	25/34	24/32	33/45	22/29	177/237
%	77	75	76	74	74	75	73	76	75
average survival days	8.3	8.8	8.0	9.8	9.0	9.3	8.3	9.0	8.9

1) Intraabdominal injections of DFP in dose of 2mg/kg plus 1mg/kg every other day.

In this experiment, 4 normal rats were also transplanted with the same ascites specimens as control. While all control rats died of tumor with typical abdominal findings, 5 treated rats were all alive and they were killed a month later but no gross pathological changes were found in the abdomen.

2) Four subcutaneous injections of DFP in does of 2mg/kg every other day.

One cases out of 6 died of poisoning after 3 injections. The remaining 5 cases were transplanted and all survived; when they were killed and autopsied, no pathological changes were found either.

From these results it was concluded that the rats with congenital resistibility were unable to be changed into susceptible rats by this premedication.

VI. DISCUSSION

ChE in serum is so-called non-specific ChE which is produced chiefly by the liver and which is different from the specific ChE which has a close relation with nervous functions. DFP exerts its action only on the non-specific ChE under certain concentration (10^{-8}-10^{-9} M)[21] but it antagonizes the specific ChE in the brain under the concentration of $10^{-6.0}$-$10^{-5.5}$ M[22] and, according to some reports, ChE value of the brain after death by injection of LD_{50} of DFP was zero, while in the survivals it was 10-20 % of normal.[10]

Clinically, DFP has vasodilator, miotic and diarrheal actions. From this point of view, a marked cholinergic domination must be produced by the DFP injection. Though some authors[23] say that changes of ChE level in serum are not parallel with those of the specific ChE, the fact that serum ChE goes down in propotrion to the dose of DFP injected shows the increase of DFP concentration in blood and it is possible that lipoid soluble DFP in blood enters into nerve tissues and nervous function will be effected.

In this paper it was found that the rate of lethal take of the YOSHIDA sarcoma increased when ChE value of serum was lowered in consequence of injecting a large dose of DFP and it was concluded that the internal circumstance where the serum ChE value was low was suitable for the tumor proliferation. The fact of the acceleration of tumor proliferation under this condition agrees, neurophysiologically, with the fact of the preferential tumor growth in the area of sympathectomy such as discussed earlier.

To exclude the death from toxity of DFP, some animals were not transplanted with the tumor after the DFP premedication and were observed as control. One case died of acute poisoning in a day after the last injection i. e., during the period of high DFP concentration, and others survived. So it was thought that toxity of DFP in these dose level could not last till it led rats to death some days later. (Table 3).

To check the possible effect of DFP on tumor transplantation, a group of negative-take which seemed to have a resistibility against the tumor growth was treated with the DFP premedication; but these animals could not be changed into susceptible ones. That is to say, the heredo-immunological condition seems to predominate over the possible effect of this treatments in the case of tumor transplantation.

Humans and animals may be divided into 3 groups, in relation to tumor, even assuming the same living circumstance.

1) One which is never affected by tumor.

2) One which recovers from tumor spontaneously or by treatment even if it were affected by tumor spontaneously or artificially.

3) One which dies of tumor in spite of the most powerful up-to-date medical treatment.

When gpoup 2) was treated with DFP, it might be changed into group 3) and the transplantation rate went up consequently; but group 1) could not be changed into group 3) by this treatment at all.

These relations have the same significance and limitation as that which hold for the X-ray irradiation and cortisone premedication to tumor transplantation.

Ⅶ. CONCLUSION

The effect of DFP upon the proliferation of the YOSHIDA sarcoma was studied and the following results were obtained.

1) ChE value in serum of normal rats was different from animal to animal and the animals were divided into 2 groups, i. e., groups of high or low ChE level.

2) The rate of lethal take of the YOSEIDA sarcoma increased significantly when ChE values of serum was lowered in consequence of injecting a large dose of DFP.

3) Negative-take-rats could not be changed into susceptible ones even when their ChE values lowered by DFP injections.

I am greatly indebted to Assist. Prof. Dr, CHUJI KIMURA of our clinic for his constant and kind help during the course of these studies.

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和 文 抄 録

神経変調組織に於ける吉田肉腫増殖についての実験的研究

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教室の井上（1959）はラツテの1側内臓神経，腎神経，腹部交感神経幹を夫々遮断して後，吉田肉腫を腹腔内に移植し，遮断側のみに腎腫瘤形成を認めている．著者は之を追試し更に之ら神経を切断して，その際の手術創が腫瘍増殖に及ぼす影響をも検査し，且つ肋間神経切断と配下領域に於ける腫瘍増殖の態度を調べて自律神経切断の場合と比較して次の結論に到達した．即ち，

1） 右側胸部交感神経幹切断，右側腹部交感神経幹遮断の後，吉田肉腫を腹腔内に移植したところ，そのうち腹部交感神経幹遮断側に28％の腎腫瘤の形成を認めた．

2） 1側内臓神経切断後に移植した例に於ては，左側切断例で33％，右側切断で29％に相当する1側性腎腫瘤形成を認め，対照側には1例も認めなかつた．

3） 腹壁に開腹創を作り第1期癒合後，腹腔内に移植すると大網癒着のない限り，腹壁手術創部に選択的な転移形成は認められなかつた．又，内臓神経切断手術創治癒後，腹腔内に墨汁を注入して，その吸収状況を調べたが，手術創部及び腎表面に特に著明な墨汁吸収の亢進を認めえなかつた．それ故に，内臓神経切断時の手術創や，注射遮断の際の損傷そのものが転移を好発したとは考え難い．

4） 腹腔内に移植した際，腫瘍性変化が最も著明に起り，且つ癒着によつて他へ転移を誘発し易い大網を故意に腹壁腹膜へ縫着してから，腫瘍を腹腔内へ移植すると，縫着部に於て大網腫瘤からの連続性転移は反つて妨害された．即ち大網縫着治癒部の瘢痕は腫瘍の連続性転移を抑制した．以上の所見から，本実験の吉田肉腫による腎腫瘤形成には，腎神経形成線維の切断が最も重要な原因をなしているもののようで，注射や手術による副損傷の影響は，腫瘤形成に対して促進的ではないと考えられる．

5） 肋間神経切除領域の皮下移植腫瘍は肋間神経切除と同時に移植した場合は，その影響は不定，切除後1週間及び1月後移植の場合は，むしろ切除側に於て抑制傾向を認めた．

次に DFP（抗コリンエステラーゼ剤）を大量に注射し，コリン性神経作用が優位な状態である場合，吉田肉腫の移植が如何なる影響を受けるかに就て調べた．

1） 健常ラッテの血清コリンエステラーゼ値（血清コ値）は各個体によつて著しい個体差があるもので，著者は便宜上，之を高値群と低値群とにした．

2） 血清コ値は DFP 注射を反復すると高値群も低値群も共に略平行して低下する．即ち DFP に対する感受性の個体差は，血清コ値によつて決るものと思われる．而も高値群のみが DFP の反復注射によく耐えた．

3） DFP を一定量以上注射してから吉田肉腫を腹腔内へ移植すると，その腫瘍による死亡率は，対照群に比べると有意の差をもつて高い．此際，同量の DFP 注射のみを行なつて腫瘍を移植しなかつた対照群では，その1例が注射後，急性中毒死をきたしたが，他は死亡しない．故に DFP の毒性による死亡率の上昇は考えられず即ち全身的にコリン性神経が優位である状態が，腫瘍増殖に好適な内部環境を呈したものと考えられ，このことは，前述の交感神経切断領域に撰択的に腫瘤を形成する傾向と一致すると云える．

4） 移植陰性のラッテに DFP 前処置を行なつたが，移植陽性には出来なかつた．故にこれらの処置による内部環境の変化よりも，先天的抗腫瘍性の方が，移植の可否を優位的に支配しているものと考えられる．

正　　誤

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Ⅱ. INTRODUCTION　は　Ⅰ. INTRODUCTION

に訂正いたします。